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Soil Biology and Biochemistry

DOI:
10.1016/j.soilbio.2018.02.004

Published: 01/05/2018

Peer reviewed version

Spatial zoning of microbial functions and plant-soil nitrogen dynamics across a riparian area in an extensively grazed livestock system

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ABSTRACT

Anthropogenic activities have significantly altered global biogeochemical nitrogen (N) cycling leading to major environmental problems such as freshwater eutrophication, biodiversity loss and enhanced greenhouse gas emissions. The soils in the riparian interface between terrestrial and aquatic ecosystems may prevent excess N from entering freshwaters (e.g. via plant uptake, microbial transformations and denitrification). Although these processes are well documented in intensively managed agroecosystems, our understanding of riparian N removal in semi-natural systems remains poor. Our aim was to assess the spatial zoning of soil microbial communities (PLFA), N cycling gene abundance (archaeal and bacterial *amoA*, *nifH*, *nirK*, *nirS*, *nosZ*), N processing rates and plant N uptake across an extensively sheep grazed riparian area. As expected, soil properties differed greatly across the riparian transect, with significant decreases in organic matter, NH_4^+, carbon (C) and N content closest to the river (< 10 m). In addition, different microbial community structures were found along the transect. The abundance of N fixation (*nifH*) increased with distance from the river (> 10 m), while ammonia oxidising archaea (AOA) increased in abundance towards the river. N_2O emissions rates were limited by C and to a lesser extent by N with greater emissions close to the river. Plant uptake of urea-derived ^{15}N was high (ca. 55-70% of that added to the soil) but 30-65% of the N was potentially lost by denitrification or leaching. Percentage recovered also suggests that the spatial patterning of plant and microbial N removal processes are different across the riparian zone. Our study provides novel insights into the underlying mechanisms controlling the spatial variability of N cycling in semi-natural riparian ecosystems.

Keywords: buffer strip, ecosystem services, DON, nitrification, heathland, wetlands.

1. Introduction

The overuse of nitrogen (N) fertilizers, alongside land use change, has caused the N saturation of many terrestrial ecosystems worldwide (Gruber and Galloway, 2008). Further, the resultant N loss from agroecosystems is contributing to many major environmental problems such as marine and freshwater eutrophication, loss of biodiversity, climate change and ecosystem acidification (Canfield et al., 2010; Erisman, 2013). Strategies are therefore needed to better retain, or sustainably remove, excess N from land under agricultural production. One potential mechanism is the active management of riparian areas at field margins to intercept and mitigate excess N from migrating towards freshwaters (Mayer et al., 2007). Within these areas, a range of interrelated biotic and abiotic processes may be involved in N attenuation, including nitrification, denitrification, mineralization, plant and microbial uptake, mass flow/diffusion and sorption-desorption (Matheson et al., 2002; Vyzamal, 2007). The importance of each process, however, is expected to vary greatly between ecosystems and also from the landscape down to the micrometre scale within the plant-microbial-soil system (Burt et al., 1999; Sanchez-Pérez et al., 2003).

Denitrification has been shown to be of particular importance for riparian wetland biogeochemistry because of the predominance of anoxic conditions, high concentrations of dissolved organic carbon (DOC) and the high rates of N fixation (Groffman and Hanson, 1997). It also represents the ultimate removal mechanism for reactive nitrogen (e.g. NO_3^-, NO_2^-, N_2O) from terrestrial and aquatic ecosystems (Seitzinger et al., 2006; Jacinthe and Vidon, 2017). In some cases, however, complete denitrification to N_2 may not occur due to a lack of N_2O reductase in the microbial community or if certain environmental conditions remain sub-optimal (e.g. soil moisture, O_2 content), leading to the potential release of environmentally damaging N_2O (Butterbach-Bahl et al., 2013).

Additionally, denitrification is strongly coupled, both spatially and temporally, with other environmental processes such as N fixation, nitrification and anaerobic ammonium oxidation (anammox) (Vyzamal, 2007; Groffman et al., 2009).

To optimise N removal by riparian areas and to implement active management, requires a good understanding of the key factors which regulate N cycling across these zones. Fundamental to this, is understanding the spatial abundance and behaviour of the underlying microbial communities which control how and when the different N transformations occur (Herbert, 1999; Chon et al., 2011). In this respect, few studies have tried to combine the analysis of key N cycling genes (abundance and transcription) and quantification of N_2O:N_2 production to gain a better insight into the spatio-temporal factors regulating N_2O fluxes (Avrahamia and Bohannan, 2009)). However, contradictory studies showing a clear relationship between gene copy number and N_2O emission rates or a total lack of it, are commonly presented, highlighting the need for further research in this area (Bakken et al., 2012; Di et al., 2014). Additionally, research in wetland biogeochemistry has frequently focused on single-ecosystem processes (i.e. denitrification) rather than providing a more holistic view of microbial community functioning (Gutknech et al., 2006). Therefore, there is a need to improve our understanding of the links (from genes to ecosystems) between physical, biogeochemical and ecological processes that drive the services of freshwater systems

Alongside the microbial community, wetland vegetation also plays a major role in regulating N losses via denitrification (Schnabel et al., 1996; Veraart et al., 2011). For example, plants can alter the size and composition of the soil microbial community, stimulate microbial activity via C rhizodeposition, and change soil oxidation status (Nijburg et al., 1997; Tabuchi et al., 2004; Groffman et al., 2009). In addition, wetland plants employ numerous physiological adaptations to overcome anoxia in waterlogged

93 soils including: shallow rooting, dumping of respiratory by-products into the rhizosphere
94 (e.g. lactic acid) and the formation of aerenchyma (Wheeler, 1999). In light of this, the
95 choice of plant species is likely to be very important for improved riparian management
96 and freshwater protection.

97 While much work has been undertaken on N removal in riparian areas adjacent to
98 intensive cropping systems, comparatively little work has been undertaken in extensively
99 grazed livestock systems (Wells et al., 2016). In these systems, urine hotspots represent
100 the major input of reactive N and are expected to greatly modify soil microbial
101 communities involved in N cycling (Di et al., 2010). In this context, the main objectives
102 of the present study were: (1) to gain further insight into the environmental factors
103 controlling riparian soil N cycling and how they contribute to explaining the spatial and
104 temporal variability of N cycling in semi-natural ecosystems; (2) to estimate the role of
105 different vegetation communities in N uptake across the riparian zone; and (3) to link N
106 cycling gene abundance to N removal processes.

107

108 **2. Materials and methods**

109 *2.1. Study site*

110 The experimental site was located in the upper, southern area of the Conwy
111 catchment, North Wales, UK (52° 59' 8.90"N, 3° 49' 15.99"W; Fig. 1; Figs. S1 and S2).
112 The study area has been classified as blanket bog according to the New Phase 1 habitat
113 survey (Lucas et al., 2011) and considered a Special Area of Conservation (SAC) under
114 the EC Habitats Directive (94/93/EEC). The climate of the upper reaches of the Conwy
115 catchment is characterized by relatively high rainfall and cool temperatures (mean annual
116 rainfall of 2180 mm and mean annual soil temperature at 30 cm depth is 8 °C; based on
117 30-year average 1981-2010 data from the UK Met Office). The area was subject to sheep

(*Ovis aries* L.) grazing at a low stocking density (0.1 ewe ha^{-1}). A detailed description of the Conwy catchment and land use can be found in Emmett et al. (2016) and Sharps et al. (2017).

2.2. Sampling strategy

Four 25 m long transects, 5-10 m apart, and perpendicular to a headwater stream of the Conwy River, were delineated for sampling during the month of October 2016 (Fig. 2). The maximum length of the transects was decided according to the extent of the riparian zone as defined by the variable buffer delineation method (de Sosa et al., 2017). Intact soil cores (5 cm diameter, 0-15 cm depth) were collected at three different zones (from this point onwards in the manuscript, these are referred to as zones 1, 2 and 3), selected according to their dominant vegetation cover (Fig. 2). Zone 1 was dominated by thick tufts of soft rush (*Juncus effusus* L.) and located < 5 m to the river. Zone 2 corresponded to the transitional area between the grasses and the heathland (5-10 m) and zone 3 (> 10 m) represented the area dominated by typical peat-forming heathland species such as bog-mosses (*Sphagnum* spp.), *Calluna vulgaris* (L.) Hull, *Erica tetralix* L. and *Scirpus cespitosus* L. (Fig. S1-S2). Along each transect, two sample points were located within zone 1 (2 and 5 m from the edge of the river), one sample point was located within zone 2 (5-10 m), and two sampling points were located in zone 3 (i.e. 15 and 25 m; Fig. 2).

Intact soil cores were taken with a Russian auger (5 cm diameter, 15 cm in length; Eijkelkamp Soil & Water, Giesbeek, The Netherlands) to conduct the main denitrification experiment. Additional intact soil cores were taken for analysis of soil physicochemical properties prior to conducting the laboratory study and a further 20 cores for bulk density determination. All soil samples were stored at 4 °C prior to analysis except for subsamples

(~25 g) which were used for Phospholipid Fatty Acid analysis (PLFA) and DNA extractions. These samples were stored immediately at -80 °C.

2.3. General soil characterization

Soil samples were passed through a 2 mm sieve to remove any plant material and to ensure sample homogeneity. They were held at field moisture for all subsequent analyses to represent field conditions. Soil water content was determined gravimetrically (24 h, 105 °C) and soil organic matter content was determined by loss-on-ignition (LOI) (450 °C, 16 h). Soil pH and electrical conductivity (EC) were measured using standard electrodes in a 1:2.5 (*w*/*v*) soil-to-deionised water mixture. Total available ammonium (NH_4-N) and nitrate (NO_3-N) in soil were determined within 0.5 M K_2SO_4 extracts (1:5 *w*/*v*) via the colorimetric salicylate procedure of Mulvaney (1996) and the vanadate method of Miranda et al. (2001), respectively. Available phosphate (P) was quantified with 0.5 M acetic acid extracts (1:5 *w*/*v*) following the ascorbic acid-molybdate blue method of Murphy and Riley (1962) and total C (TC) and N (TN) were determined with a TruSpec® elemental analyser (Leco Corp., St Joseph, MI). Dissolved organic C (DOC) and total dissolved N (TDN) were quantified in 1:5 (*w*/*v*) soil-to-0.5 M K_2SO_4 extracts (Jones and Willett, 2006) using a Multi N/C 2100 TOC analyzer (AnalytikJena, Jena, Germany). Total soil porosity was determined using the equation of 1-(bulk density/particle density for organic soils) and percent water-filled pore space (WFPS) was obtained from the relationship between the volumetric water content and total soil porosity. Anaerobic mineralizable N (AMN) was determined by the anaerobic incubation of soil samples for 14 days at 25-30 °C in the dark, followed by extraction with 1 M KCl and measurement of NH_4-N produced as described above (Bundy and Meisinger, 1994). Anaerobically mineralizable organic C (AMOC) was calculated as described in Ullah and

Faulkner (2006). Briefly, moist soil samples were placed in gas-tight containers and NO_3^- was added to remove any soil limitation. Containers were purged with N_2 gas to induce anoxic conditions and stored in the dark at room temperature (25 ºC). The headspace of the containers was sampled after 1, 24, 48 and 72 h of incubation and analysed for CO_2 concentration on a Clarus 500 gas chromatograph with a TurboMatrix headspace autoanalyzer (Perkin-Elmer Inc., Waltham, CT).

2.4. Phospholipid fatty acid analysis

Microbial community structure was measured by phospholipid fatty acid (PLFA) analysis following the method of Buyer and Sasser (2012). Briefly, samples (2 g) were freeze-dried and Bligh-Dyer extractant (4.0 ml) containing an internal standard added. Tubes were sonicated in an ultrasonic bath for 10 min at room temperature before rotating end-over-end for 2 h. After centrifuging (10 min) the liquid phase was transferred to clean 13 mm × 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water added. The upper phase was removed by aspiration and discarded while the lower phase, containing the extracted lipids, was evaporated at 30 °C. Lipid classes were separated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA). Phospholipids were eluted with 0.5 ml of 5:5:1 methanol:chloroform:H_2O (Findlay, 2004) into glass vials, the solution evaporated (70 °C, 30 min). Transesterification reagent (0.2 ml) was added to each vial, sealed and incubated (37 °C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were then added. The chloroform was evaporated just to dryness and the samples dissolved in hexane. The samples were analysed with a 6890 gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with autosampler, split-splitless inlet, and flame ionization detector. Fatty acid methyl esters were separated on an Agilent Ultra 2

193 column, 25 m long × 0.2 mm internal diameter × 0.33 µm film thickness. Standard
194 nomenclature was followed for fatty acids (Frostegård et al., 1993). A detailed description
195 of PLFA markers and taxonomic microbial groups is provided in Table S1.
196
197 *2.5. Denitrification and potential N_2O emissions*
198 Denitrification rates were measured using the acetylene (C_2H_2) block method based
199 on the intact core technique developed by Tiedje et al. (1989). Although this technique
200 presents limitations such as the poor diffusion of C_2H_2 into the soil, it has been found to
201 produce similar results to experiments using ^{15}N tracers (Aulakh et al., 1991).
202 In brief, intact soil cores (approximately 37 ± 1.5 g dry weight soil) were placed in
203 PVC tubes (10 × 15 cm) to maintain soil structure. These tubes were then placed in gas-
204 tight containers (1.4 dm^3 volume; Lock & Lock Ltd., Seoul, Republic of South Korea).
205 To measure denitrification, 20 ml of 4 different C and N amendments were applied
206 to individual soil cores (n = 20 per amendment):
207 1) Control (distilled water addition only)
208 2) Glucose-C addition (glucose solution containing 4 g C l^{-1}; 55 mM glucose)
209 3) Urea-N addition (artificial sheep urine containing 2 g N l^{-1}; Selbie et al., 2015)
210 4) Urea-N + glucose-C addition (artificial urine plus glucose solution containing 2
211 g N l^{-1} and 4 g C l^{-1} respectively).
212 Urea was selected as it represents one of the main N inputs to upland grazed ecosystems.
213 The N concentration was chosen according to the concentration range in urine under a
214 light grazing regime (Selbie et al., 2015). The ratio of C-to-N was chosen based on
215 experimental values presented in Her and Huang (1995). Glucose was chosen as it
216 represents a labile C substrate that can be utilized by almost all soil microorganisms

(Gunina and Kuzyakov, 2015). The concentration of added C also reflects a typical sugar concentration that would occur in soil upon root cell lysis (Jones and Darrah, 1996).

All cores were directly injected with 5 ml of C_2H_2 into the middle of the soil volume. The cores were then placed into gas-tight containers and 10% of the headspace replaced with C_2H_2 to block the conversion of N_2O to N_2 gas. The control cores were only amended with 20 ml of distilled water without C_2H_2 addition. The containers were stored at 10 ºC in the dark to prevent C_2H_2 breakdown. Headspace gas was sampled at 0, 2, 6 and 24 h and stored in pre-evacuated 20 ml glass vials before being analysed for N_2O concentration on a Clarus 500 gas chromatograph with a TurboMatrix headspace autoanalyzer (Perkin-Elmer Inc., Waltham, CT). Prior to gas sampling, the headspace was homogenised by gently mixing with a syringe. At the end of the experiment, each individual core was weighed and N_2O fluxes corrected accordingly. The rate of N_2O production was calculated in μg $N\text{-}N_2O$ g^{-1} dw h^{-1}. Cumulative N_2O emissions were calculated by integration using the trapezoidal rule.

2.6. Nitrogen uptake by vegetation

The role of vegetation in N uptake was measured in the field using ^{15}N-labelled urine. Two independent sets of plots (50 cm times 50 cm) were randomly selected within each replicate vegetation zone, one set received no N additions (herein referred to as the control set) while the second received ^{15}N-labelled artificial urine.

Prior to addition of the ^{15}N-labelled treatment, turfs (20 cm times 20 cm) and associated soil (0-15 cm depth) were taken from the centre of each of the control plots to obtain ^{15}N natural abundances for each plant and soil component. After harvest, the samples were transferred to the laboratory and separated into soil, roots, shoots and mosses for ^{15}N determination. Subsequently, in each ^{15}N-labelling plot, 250 ml of

242 artificial urine labelled with ^{15}N urea (15 atom %) at a rate of 2 g N l^{-1} was applied
243 (equivalent to 20 kg N ha^{-1}). Ten pulses of ^{15}N-labelled urine (each pulse was 25 ml in
244 volume) were injected with a syringe (0.84 mm bore × 5 cm long) into the soil underneath
245 the plants (0-15 cm depth) in the centre of the plot. Depth of urine injection was selected
246 according to previous observations of urine infiltration into the soil. The volume and
247 concentration of N added followed that of a typical sheep urine event (Marsden et al.,
248 2016). Immediately after the final ^{15}N pulse addition, the area was protected with
249 individual wire mesh cages to prevent livestock trampling and grazing. One week after
250 ^{15}N addition, a 20 × 20 cm^2 turf and associated soil (0-15 cm depth) was harvested from
251 the middle of each plot, transferred to the laboratory and separated into soil and plant
252 components as described above for ^{15}N determination.

253 Soil for ^{15}N analysis was passed through a 2-mm sieve and subsamples (ca. 40 g)
254 were oven-dried (48 h, 80 °C) before being weighed and ground for ^{15}N analysis. Plant
255 shoot and root material followed the same drying procedure after being washed with
256 distilled water to remove any exogenous isotope label. The same procedures were
257 followed for the control samples one week before to avoid any cross-contamination with
258 the ^{15}N-urea labelled samples. All fractions were analysed separately for $\delta^{15}N$ at the UC
259 Davis Stable Isotope Facility (UC Davis, Davis, CA). Values of ^{15}N are presented directly
260 as the atom% of ^{15}N in the sample. The ^{15}N atom% excess was calculated as the ^{15}N
261 atom% difference between enriched samples and values of background natural
262 abundances (control). Recovery of tracer ^{15}N (%) was calculated by multiplying the N
263 content in the pool by it mass per square meter and ^{15}N atom% excess divided by total
264 added ^{15}N per square meter (Xu et al., 2011).

265

266 *2.7. DNA extraction and quantitative PCR*

A subsample of soil (ca. 25 g) was taken from each of the cores used for physicochemical analysis and stored at -80 °C prior to DNA extraction. The DNA was extracted from three 250 mg subsamples using an UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions. Triplicate DNA extractions for each soil sample were pooled together to give a total volume of 150 µl. Extractions of DNA were concentrated to give a final volume of 50 µl using a Savant SVC100H SpeedVac Concentrator (ThermoFisher Scientific Inc., Waltham, MA). Extracted DNA was visualized by 0.9% agarose gel electrophoresis and nucleic acid staining with SafeView® (NBS Biologicals, Huntingdon, UK). The concentrations of DNA where checked using Quant-iT™ dsDNA Assay Kit (ThermoFisher). Samples were then stored at -80 °C prior to further analysis.

Microbial N cycling gene abundance was investigated by quantitative-PCR (qPCR) targeting specific genes or genetic regions. Bacterial and archaeal communities were targeted via the 16S rRNA genes, while the fungal community abundance by the ITS region. The different communities involved in N-cycling were investigated: N fixation (*nifH* gene); nitrification by targeting the ammonia oxidising bacteria (AOB) and archaea (AOA) (*amoA* gene), and denitrifiers via the nitrite reductase (*nirK* and *nirS* genes) and the nitrous oxide reductase (*nosZ* genes clade I and II) (Table S2).

Quantitative-PCR amplifications were performed in 10 µl volumes containing 5 µl of QuantiFast (Qiagen, Manchester, UK), 2.8 µl of nuclease-free water (Severn Biotech, Kidderminster, UK), 0.1 µl of each primer (1 µM) and 2 µl of template DNA at 5 ng μl^{-1}, using a CFX384 Touch® Real-Time PCR Detection System (Bio-Rad, Hemel Hempstead, UK). The standards for each molecular target were obtained using a 10-fold serial dilution of PCR products amplified from an environmental reference DNA (also used as positive control) and purified by gel extraction using the Wizard® SV Gel and

292 PCR Clean Up System (Promega, Southampton, UK) following the manufacturer's
293 instruction and quantified by fluorometer Qubit® 2.0 dsDNA BR Assay Kit (Thermo
294 Fisher Scientific). Standard curve template DNA and the negative/positive controls were
295 amplified in triplicate. Amplification conditions for all qPCR assays consisted in 2 steps:
296 first denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 10 s and 60 °C
297 for 30 s that included annealing, elongation and reading. Each amplification was followed
298 by melting curve (increase in temperature from 60 °C to 95 °C, with a reading every 0.5
299 °C) to assess the specificity of each assay. The efficiency of the qPCR varied between
300 81.5% and 94.5%, and r^2 between 0.996 and 0.999. The melting curves showed specificity
301 for all the genes, except as expected for the fungal ITS, that showed the amplification of
302 products of different lengths, due to the variability in length of the ITS region between
303 different fungal taxa (Manter and Vivanco, 2007).
304
305 *2.8. Statistical analysis*
306 Statistical analysis was performed with SPSS v22 for Windows (IBM Corp.,
307 Armonk, NY). All data were analysed for normality and homogeneity of variance with
308 Shapiro Wilk's tests and Levene's statistics, respectively. Transformations (log_{10} or
309 square root) to accomplish normality and homogeneity of variance were done when
310 necessary (i.e. bulk density, available P, microbial biomass PLFA, ^{15}N recovery, and
311 cumulative N_2O, untransformed values are presented). For all statistical tests, $P < 0.05$ was
312 selected as the significance cut-off value. Analysis of variance (one-way ANOVA) was
313 performed to explore the difference of soil physicochemical properties, gene copy
314 numbers, PLFA ratios of microbial groups respective to distance from the river followed
315 by Tukey's post-hoc test to assess differences across the riparian transect. Principal
316 component analysis (PCA) was used to explore the spatial relationships of PLFA

317 microbial groups (%) relative to distance from the river. Cumulative N_2O emissions after
318 treatment application across the riparian transect were compared by Welch's test followed
319 by Games-Howell post-hoc test, due to the data not conforming to homogeneity of
320 variance even after data transformation. In contrast, a one-way ANOVA followed by
321 Tukey's post-hoc test was performed to assess differences in cumulative N_2O emissions
322 between treatments for each sampling distance from the river (i.e. 2, 5, 10, 15 and 25 m).
323 Two separate analyses were conducted to explore differences in ^{15}N recovery due to the
324 data not conforming to homogeneity of variance even after data transformation. A one-
325 way ANOVA and Tukey's post-hoc test was performed to explore differences in the
326 percentage allocation of ^{15}N to the different fractions (e.g. shoots, roots, moss and soil)
327 across the different riparian zones. A second one was used to assess how ^{15}N recovery
328 differed within each specific fraction across the three zones. A mixed model was also
329 performed with distance from the river as a fixed effect and transect as a random effect
330 to assess ^{15}N recovery and gene copy number across the riparian zone, but the results did
331 not differ from ANOVA, and only the ANOVA results is presented in the article

332 Spearman rank correlation coefficients (ρ) were used to evaluate the relationship
333 between soil physicochemical properties and cumulative N_2O emissions, gene copy
334 number, or PLFA biomarkers ratio whereas linear regressions (r^2) were used between soil
335 physicochemical properties and PLFA biomarker ratios.
336

337 **3. Results**

338 *3.1. General soil characterization*

339 Significant differences in all soil properties, except for NO_3^- and total dissolved N
340 concentration and anaerobic mineralizable N (AMN), were found across the riparian
341 transect relative to distance from the main river channel (Table 1). Zone 2 showed an

increase in pH values by 0.66-0.85 unit in comparison with zone 1 and zone 3. Likewise, EC was approximately 2-fold greater in zone 1 and 3 relative to zone 2. In addition, soil organic matter (SOM) tended to increase with distance from the river being 60% higher at the distal points (15 and 25 m) compared with those closer to the river. The high SOM levels associated with soils furthest away from the river contributed to lower bulk densities, higher soil porosities and increased soil water content. Available NH_4^+ concentrations were 3.6 greater in soil from zone 3 in comparison with zone 1 and 1.8 times greater than the soil in zone 2, while NO_3^- did not show any significant differences. Similarly, available P was 10-times greater in zone 3 relative to zones 1 and 2. Total C, total N and the C-to-N ratio were greater in zone 3 relative to zones 1 and 2 and a similar trend was also observed for DOC.

Anaerobic incubation of soils across the transect showed that the amount of AMOC in zone 3 was significantly greater than in zone 1 and 2 (~ 3 and 1.5 times, respectively) (Table 1). In contrast, AMN showed little trend across the transect.

3.2 Microbial community structure and abundance

Microbial biomass determined from total PLFA content showed a general decline across the riparian transect towards the river channel. Principal Component Analysis (PCA) of PLFA microbial groups (% abundance) across the transect explained 72.6% of the total variance within the dataset on the first two principal components (PC) (Fig. 3). The spatial segregation of cluster centroids within the PCA indicates that in zone 1 the most influential components were anaerobes and putative arbuscular mycorrhizal fungi (AM fungi). In contrast, Gram (+) and Gram (-) bacteria were the dominant groups in zone 2 and 3, respectively. Zone 2 showed the greatest microbial variability.

366 The fungi/bacteria ratio decreased by 2 to 2.5 times from zone 1 to zone 2 and 3
367 (Table S3, $P = 0.008$). The ratio of Gram (+)/Gram (-) was over 2 times greater in zone 2
368 than zone 3 but it did not differ from zone 1 ($P = 0.001$). On average, 16w/17cyclo and
369 18w/19cyclo ratio (indicative of an actively growing community under low stress
370 conditions) was 2.5-fold greater in zone 3 than zone 1 and 2 (Table S3, $P < 0.0001$). There
371 were highly positive relationships between fungi/bacteria ratio with bulk density and
372 negatively with total porosity and soil water content ($r^2 = 0.60$, $P < 0.001$ for bulk density
373 and total porosity, $r^2 = 0.45$, $P = 0.001$, soil water) whereas Gram (+)/Gram (-) ratio was
374 negatively correlated to NH_4-N, soil water content, SOM, available-P and DOC content
375 ($r^2 > 0.68$ for available P and DOC, $r^2 > 0.53$ the rest, $P < 0.001$ in all cases). In contrast,
376 a positive correlation was found between 16w/17cyclo and 18w/19cyclo ratios and DOC,
377 soil water, C-to-N ratio and SOM content ($r^2 > 0.61$, for DOC, $r^2 > 0.71$, for soil water
378 and C-to-N ratio, $r^2 > 0.82$, for SOM, $P < 0.001$ in all cases).
379 The archaeal 16S rRNA gene abundance tended to increase with distance from the
380 river but the results were not significantly different ($P > 0.05$) (Fig. 4). In contrast, the
381 fungal ITS region abundance showed the opposite trend but was also not significant. The
382 bacterial 16S rRNA gene abundance displayed on average 2 times greater bacterial copies
383 in zone 2 than the distal area but it was not significant (Fig. 4).
384 Significant positive correlations were found between bacterial *16SrRNA* and pH
385 and EC ($\rho = 0.48$, -0.45, respectively) whereas archaeal *16SrRNA* correlated negatively
386 with soil bulk density and positively with total porosity ($\rho = -0.57$, 0.56, respectively;
387 Table 2).
388
389 *3.2. ^{15}N uptake by the vegetation*

No significant differences were found between the recovery of ^{15}N in the different plant and soil fractions across the riparian transect (zone 1, 2 and 3; $P > 0.05$). Similar percentages of total ^{15}N recovery of added ^{15}N were obtained for plants and soil in zones 2 and 3 (71.9 % and 79.3%, respectively), whereas only 56.8% was recovered in the plants and soil within zone 1 although it was not significant (Fig. 5). Generally, there were very few differences between the amounts of ^{15}N recovered in the different plant-soil fractions within each zone, Only in zone 2, were four times more ^{15}N was recovered in the shoots compared to the soil ($P = 0.012$; Fig. 5).

3.3. Potential denitrification and N_2O emissions

In response to the addition of labile C and/or N to the soil, greater cumulative N_2O emissions were only observed within zone 1, showing little or no effect in zones 2 and 3 (Fig. 6). In zone 1, the addition of labile C-only increased N_2O emissions by a factor of 1000, from 0.004 ± 0.001 to 4.07 ± 0.14 mg N kg^{-1} h^{-1} relative to the control in the area closest to the river (e.g. 2 m, $P < 0.001$). Similarly, the addition of C and N together also increased N_2O emissions relative to the control (0.004 ± 0.001 to 2.95 ± 0.14 mg N kg^{-1} h^{-1}) at 2 m from the river. After the addition of labile C alone or in combination with N, emissions of N_2O were 78 and 45 times higher, respectively than the control at 5 m from the river (Fig. 6). Although urea-N addition also increased N_2O emissions in zone 1 (0.24 ± 0.06 mg N kg^{-1} h^{-1} at 2 m, and 0.61 ± 0.36 mg N kg^{-1} h^{-1} at 5 m), fluxes were not significantly different from the control ($P > 0.05$).

N_2O emissions across the riparian transect significantly differed for all treatments with respect to the distance from the river ($P < 0.001$, treatments with C addition alone or in combination with N addition; $P < 0.05$, urea-N only addition). Basal emissions of N_2O from the control cores did not show significant differences with distance from the

415 river ($P > 0.05$). Carbon-only addition greatly stimulated emissions of N_2O with distance
416 from river, with the area closest to the river (2 m) emitting on average 80 times more N_2O
417 than the distal point of the transect (25 m). The addition of C together with N increased
418 N_2O emissions at 2 m from the river by 60, 90 and 101% in comparison to the amount
419 emitted at 5 m and zone 2 and 3, respectively (Fig. 6).

420 Significant positive correlations were found between N_2O emissions and bulk
421 density, whereas soil water content, total N, total porosity and AMOC correlated
422 significantly but negatively with N_2O production for all treatments except the control
423 (Table 3).

424

425 *3.4. N cycling gene abundance*

426 Ammonia oxidizing bacteria (AOB) and archaea (AOA) showed different
427 abundance patterns with respect to distance from the river (Fig. 7). While the proximity
428 of the river had no effect on the bacterial *amoA* gene numbers, archaeal *amoA* gene copy
429 number significantly decreased ($P = 0.001$) on average by up to 84% from zone 1 closest
430 to the river to zone 2 and by 98% with respect to zone 3. The archaeal-to-bacterial *amoA*
431 gene ratios were approximately 5 and 46-fold greater in zone 1 relative to zone 2 and 3
432 respectively (Fig. S3). In contrast, the *nifH* gene abundance significantly increased ($P =$
433 0.001) from close to the river to the distal point by 67-82%, whereas a difference with
434 respect to zone 2 was only found for 2 m. Zone 1, specifically the closest point to the
435 river, displayed the lowest value for *nirS* gene abundance which represents 3.5 lower
436 values than zone 2 ($P = 0.038$) (Fig. 7). In contrast, *nirK* and *nosZ* gene copy numbers
437 did not change significantly across the transect ($P > 0.05$). The clade II of the *nosZ* gene
438 could not be amplified despite the positive control being amplified (data not shown)

Abundance of *nirS* and *nosZ* genes correlated positively with pH (ρ ~0.5) but negatively with EC (ρ = -0.52, -0.63, respectively) (Table 2). A negative correlation was found between *nifH* and soil bulk density while archaeal *amoA* was correlated positively with bulk density (Table 2). Significant positive correlations were found between *nifH* and soil water content, AMOC, total porosity, NH_4^+ content and microbial PLFA whereas archaeal *amoA* abundance correlated negatively to the same soil properties (Table 2). The bacterial *amoA* and *nirS* genes did not show any significant correlations.

A positive strong correlation was found between copies of bacterial *16SrRNA*, bacterial *amoA* and *nirK* ($\rho > 0.73$, $P < 0.001$ in all cases) whereas *nifH* showed a highly positive correlation with *nirK* ($\rho > 0.52$, $P = 0.001$).

4. Discussion

4.1. Soil biology and biogeochemistry across the riparian zone

The riparian zone showed distinct spatial patterns in soil properties, despite the relatively short length of the transect. Results from this study clearly showed that vegetation, influenced in turn by the prevailing hydrodynamic conditions, had a striking effect on most of the soil's physicochemical properties. This finding is supported by a range of studies which have established that mean high water level together with the frequency of water fluctuation is a critical factor controlling species diversity and abundance close to watercourses (Wierda et al., 1997; Lou et al., 2016). In our study, there were lower amounts of soil organic matter and nutrients (N and P) in soils close to the river in comparison to those further away. These can be ascribed to differences in erosion-depositional processes occurring along the transect. Alongside differences in water table depth, this has led to the formation of two very distinct vegetation communities: one that contains species that can tolerate extreme waterlogging and anoxia

(via aerenchyma formation and organic acid excretion) and high levels of exogenous Fe^{2+} and Mn^{2+} (e.g. *Juncus effusus*; Visser et al., 2006; Blossfeld et al., 2011), and another that relies on obligate aerobic symbionts, which lacks aerenchyma and can only tolerate mild hypoxia (e.g. *Calluna* heathland; Gerdol et al., 2004; Rydin and Jeglum, 2013). These differences in plants are likely to be a key driver in shaping rhizosphere microbial communities and the dominant N cycling pathways.

The microbial community structure was different in the three riparian zones due the distinct soil physicochemical properties, and plant cover that are highly dependent on local hydrological regime (Gutknecht et al., 2006; Balasooriya et al., 2007). For example, the fungal-to-bacterial ratio was very low indicating a clear dominance of bacteria community over fungi. Nevertheless, the higher ratios in areas close to the river suggests a zonation pattern in fungal communities across the transect, probably linked to plant type and poor nutrient conditions (Bohrer et al., 2004; Six et al., 2006). The Gram (+)/Gram (-) ratio decreased in zone 3 (≥ 15 m) in relation to the increase in SOM, total C and N content. Gram (-) bacteria are thought to be copiotrophic organisms with a high growth rate, using labile substrate such as in zone 3, while Gram (+) bacteria are thought to be oligotrophic organisms that are better decomposers of less labile soil organic matter but have a lower growth rate (Fierer et al., 2007). Furthermore, the greater relative abundance of cyclopropanes close to the river (64% more than distal areas), which indicates the growth rate in the bacterial community and has been linked to changes in nutrient availability, infers that the most rapid growth or turnover rates will occur in distal areas of the river as a result of higher nutrient availability and lower stress conditions (i.e. water fluctuation) (Ponder and Tadros, 2002; Bossio et al., 2006).

4.2. N cycling across the riparian transect

The balance between the different steps of the N cycle varied along the riparian transect, while the plant and soil retention potential was constant, showing the varying potential of riparian wetland for N attenuation. The amount of N added did not exceed N plant demand, however, the total higher plant recovery of ^{15}N (ca. 30-40%) indicated a relatively high rate of removal. A similar amount of N was retained in the moss layer or soil (either in solution, sorbed to the solid phase, or immobilized in the microbial biomass) indicating that approximately 30-65% was lost by denitrification (as NO, N_2O or N_2), mass water flow, or translocated by roots out of the ^{15}N addition area. Our results are consistent with short-term ^{15}N recovery by vegetation in other non-riparian studies (e.g. grasslands; Nordbakken et al., 2003; Wilkinson et al., 2015). However, the high variability in ^{15}N recovery between replicates, most likely due to inherent heterogeneity in riparian areas, made it difficult to identify any consistent spatial patterns in N uptake across the riparian transect (Williams et al., 2015). Additionally, only short-term fate of urea-N was studied and differences in mass flow under the vegetation (and therefore ^{15}N residence time) was not accounted for (Weaver et al., 2001).

The genes abundance of the different steps of the N cycle showed niche differentiation along the riparian transect. The *nifH* gene spatial distribution showed a strong link to areas with lower soil water content, bulk density and higher porosity and NH_4^+ concentrations, indicating the potential role of N fixation in zones 2 and 3 to accumulate NH_4^+ in soil. This is consistent with these plant communities (e.g. *Calluna-Eriophorum* and *Sphagnum* species) being severely N limited (Leppanen et al., 2015). In contrast, AOA abundance followed the opposite trend than *nifH* gene, with the same factors explaining their distribution. Thus, we conclude that nitrogen fixation and nitrification are not coupled in the riparian wetland. This also implies that the archaea are the main microorganisms involved in nitrification over bacteria (Caffrey et al., 2007;

514 Erguder et al., 2009). Thus, despite AOA and AOB delivering the same function, the two
515 communities live in distinct niches with different drivers. The low abundance of AOB is
516 likely due to the low soil pH (4.05 – 4.90), that favour AOA (Leininger et al., 2006),
517 while the drop in AOA abundance in distal zone could be related to the higher
518 concentration of NH_4^+ (Verhamme et al., 2011) or the change in soil water content.

519 Thus, the variation in ammonia oxidisers along the riparian transect will directly
520 affect the rate of denitrification. The constant NO_3^- concentration along the transect,
521 indicate that denitrification is occurring close to the river, which was confirmed by the
522 potential denitrification rates, highly stimulated by C addition (glucose), and to a lesser
523 extent by N (urea) in this area. It is well established that denitrification rates are usually
524 enhanced by anoxic conditions, high NO_3^- availability and labile organic C (Weier et al.,
525 1993). This is supported by the oligotrophic nature of the habitat, the high C-to-N ratio
526 of the soil, and the recalcitrant nature of the plant litter produced by the vegetation (Witt
527 and Setala, 2010). Although the *Calluna* heath soil possessed high levels of DOC, this
528 has previously been shown to be largely resistant to microbial attack due to its high
529 aromatic content (Stutter et al., 2013). Interestingly, N_2O production was stimulated
530 greatly in the *Juncus effusus* zone when labile C was added, however, there was not a
531 cumulative effect after the addition of C and N together. The low concentrations of NO_3^-
532 in this zone also suggests that any NO_3^- produced could be lost to the river or is absorbed
533 by plants. Overall, nitrification appears to be the rate limiting step in N cycling within the
534 riparian wetland studied here.

535 With respect to the functional genes of the denitrifier community, none of the
536 genes studied showed high abundance close to the river. Only the *nirS* gene displayed a
537 higher abundance within zone 2, related to the increase in soil pH by less than a pH unit,
538 highlighting the sensitivity of *nirS* gene abundance to pH (Liu et al., 2010). However, the

relatively higher abundance of *nirS* was not translated into higher N_2O, although it should be noted that *nirS* and *nirK* code for nitrite reductase. The fungi, could also play a role in the denitrification as they possess *nirK* and *nirS* genes, which were not captured by the primers used. Some studies have indicated that N_2O emissions from fungal communities can be significant as they lack the *nosZ* gene to reduce N_2O to N_2; their contribution in riparian areas remains uncertain and further work is needed to explore their role further (Ma et al., 2008; Seo and DeLaune, 2010).

It is difficult to conclude on the potential N_2O emissions because the acetylene assay used in the study block the reduction of N_2O into N_2. The higher N_2O emissions close to the river after C and N addition could then be reduced. However, the constant *nosZ* clade I gene abundance and the absence of *nosZ* clade II gene along the transect, might indicate that N_2O is more likely to be emitted from the area close to the river, while the distal zone might be a sink for N_2O. Therefore, from a management perspective, restricted access to grazing and OM amendments which are commonly used for wetland restoration to accelerate soil development and regulate soil moisture fluctuation, would be recommended to avoid future potential greenhouse gas emissions in wetlands under grazing regimes (Bruland et al., 2009).

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5. Conclusions

In terms of preventing freshwater pollution, riparian areas represent one of the most valuable management tools for preventing excess nutrient loss from land to water. Most studies to date, however, have focused on N and P cycling and transformations in riparian soils adjacent to arable and intensively managed grasslands. Given the heterogeneous nature of land use in many catchments, and the trend towards modelling ecosystem services at the catchment scale, we need to gain a better understanding of

riparian N transformations across a variety of habitats and under different land use intensities. Our study in an extensively managed agricultural system clearly showed that changes in environmental factors such as breaks in vegetation or soil water saturation provide strong indicators of the relative importance of different biotic and abiotic processes involved in N cycling. However, our results also revealed hidden gradients in microbial community structure and N cycling gene abundance across the riparian strip. This reflects differences in key soil properties (e.g. organic matter content, redox) and also possibly the source of nutrients flowing through the soil (i.e. in hyporheic water flow versus lateral flow from upslope areas) and N_2O fluxes. This type of spatial information can be used for more accurate mapping of ecosystem services at the catchment scale and the design of better livestock management systems (e.g. prevention of grazing in riparian areas to avoid N_2O emissions). While we have provided novel insights into the dominant pathways for N removal in riparian zones, further work is required to investigate if seasonal patterns exist and how closely gene abundance is related to gene expression.

Acknowledgements

This research was supported by the UK Natural Environment Research Council under the Macronutrients Programme from a NERC grant: NE/J011967/1: The Multi-Scale Response of Water Quality, Biodiversity and Carbon Sequestration to Coupled Macronutrient Cycling from Source to Sea. This research was also supported by a Knowledge Economy Skills Scholarship (KESS 2) awarded to LDS funded via the European Social Fund (ESF) through the European Union's Convergence program administered by the Welsh Government.

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805

Figure Legends

Fig. 1. The Conwy catchment, North Wales, UK showing the location of the riparian sampling area and the major land cover classes.

Fig. 2. Location of sample points across the riparian area. Different colours indicate changes in vegetation. Zone 1 represents the area dominated by *Juncus effusus*, Zone 2 corresponds to the transitional area between the grasses and the heath, and Zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species.

Fig. 3. Correlation bi-plot from the principal component analysis (PCA) on PLFA microbial groups (%) with respect to distance from the river (n = 4). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (2 and 5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m), and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (15 and 25 m). Correlation of PLFA microbial groups with the main axes are given by their specific names and distance from the river by cluster centroids (average score on each horizontal principal component (PC1) and vertical principal component (PC2) with standards errors). Circles represents sample points within the same zone.

Fig. 4. Total bacterial, archaeal and fungal gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences ($P > 0.05$) with respect to distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values (n = 4 for 10, 15 and 25 m, n = 3 for 2 m and n = 2 for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 2.

Fig. 5. Recovery of ^{15}N (% of total applied) from within the different fractions (shoots, roots, mosses and soil) represented by bars (n = 3 except moss in zone 1 where n = 1). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m) and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (25 m). Same lower case letters indicate no significant differences (P > 0.05) with respect to the different fractions within each zone according to one-way ANOVA and Tukey post-hoc test.

Fig. 6. Cumulative N_2O emissions via denitrification in unamended soil (control) or after the application of labile C (glucose) and N (urea) either alone or in combination. Same lower case letters indicate no significant differences (P > 0.05) with respect to distance from the river according to Welch's test and the Games-Howell post-hoc test. Same capital letters indicate no significant differences (P > 0.05) between treatments for each distance from the river according to one-way ANOVA and Tukey post-hoc test. Bars represent mean values (n = 4) ± SEM.

Fig. 7. Bacterial *amoA* (AOB), archaeal *amoA* (AOA), *nifH*, *nirS*, *nosK*, *nosZ* gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences (P > 0.05) relative to distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values (n = 4 for 10, 15 and 25 m, n = 3 for 2 m and n = 2 for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 2.

Research Highlights

- Microbial community structure changed with distance from the river.
- *amoA* gene abundance increased towards the river while *nifH* decreased.
- N_2O emissions rates were C limited but were greatest close to the river.
- Plant uptake of urea-^{15}N was high across the riparian zone.
- The spatial pattern of N removal by riparian plants and microbes was different.

Table 1. Soil physicochemical properties across the riparian transect. Different zones indicate changes in vegetation community with zone 1 being closest to the river. Values represent means ± SEM (n = 4). Same lower-case letters indicate no significant differences ($P > 0.05$) with regard to distance from river according to One-way ANOVA and Tukey or Games-Howell post-hoc test. Results are expressed on a soil dry weight basis.

Soil property	Zone 1		Zone 2	Zone 3	
	2 m	5 m	10 m	15 m	25 m
pH	4.18 ± 0.08^{a}	4.24 ± 0.05^{a}	4.90 ± 0.09^{b}	4.12 ± 0.02^{a}	4.05 ± 0.01^{a}
EC (µS cm^{-1})	23.4 ± 3.2^{a}	21.1 ± 2.0^{a}	11.6 ± 1.0^{b}	23.3 ± 2.4^{a}	26.3 ± 1.8^{a}
Bulk density (g cm^{-3})	0.31 ± 0.019^{a}	0.20 ± 0.026^{a}	0.09 ± 0.005^{b}	0.09 ± 0.004^{b}	0.09 ± 0.008^{b}
Total porosity (cm^3 cm^{-3})	0.78 ± 1.33^{a}	0.86 ± 1.88^{ab}	0.94 ± 0.36^{b}	0.94 ± 0.32^{b}	0.93 ± 0.54^{b}
Soil gravimetric water content (g kg^{-1} soil)	659 ± 28^{a}	720 ± 5^{a}	793 ± 34^{a}	892 ± 2^{b}	899 ± 0.6^{b}
Organic matter (g kg^{-1} soil)	364 ± 20^{a}	470 ± 12^{b}	542 ± 87^{ab}	953 ± 5^{c}	965 ± 4^{c}
NH_4^+-N (mg kg^{-1} soil)	5.06 ± 0.95^{a}	4.75 ± 0.70^{a}	9.50 ± 1.56^{ab}	18.5 ± 1.94^{b}	16.7 ± 3.43^{b}
NO_3-N (mg kg^{-1} soil)	9.38 ± 0.92^{a}	12.6 ± 2.40^{a}	8.12 ± 3.09^{a}	10.5 ± 1.95^{a}	8.00 ± 0.91^{a}
Available P (mg kg^{-1} soil)	5.82 ± 3.60^{a}	3.10 ± 1.11^{a}	5.99 ± 3.68^{a}	56.0 ± 10.1^{b}	50.5 ± 13.7^{b}
Total C (g kg^{-1} soil)	215 ± 9^{a}	281 ± 8^{b}	330 ± 57^{abc}	576 ± 4^{c}	588 ± 17^{c}
Total N (g kg^{-1} soil)	8.58 ± 0.61^{a}	12.0 ± 0.57^{b}	15.5 ± 2.58$^{ab}_{c}$	17.1 ± 0.11^{c}	15.7 ± 0.38^{c}
C-to-N ratio	25.3 ± 0.77^{a}	23.5 ± 0.92^{ab}	21.3 ± 0.53^{b}	33.8 ± 0.32^{c}	37.0 ± 1.96^{c}
Dissolved organic C (g kg^{-1} soil)	0.24 ± 0.02^{a}	0.36 ± 0.02^{bc}	0.38 ± 0.07^{ab}	1.31 ± 0.18^{cd}	1.09 ± 0.14^{cd}
Total dissolved N (g kg^{-1} soil)	0.04 ± 0.005^{a}	0.05 ± 0.005^{a}	0.06 ± 0.009^{a}	0.44 ± 0.29^{a}	0.11 ± 0.025^{a}
Microbial biomass PLFA (mmol kg^{-1} soil)	1.12 ± 0.21^{a}	2.02 ± 0.27^{a}	3.83 ± 1.25^{ab}	7.58 ± 0.54^{b}	7.29 ± 1.70^{b}
AMOC (mg C-CO_2 kg^{-1} soil h^{-1})	0.23 ± 0.04^{a}	0.41 ± 0.06^{ab}	0.61 ± 0.16^{ab}	0.92 ± 0.14^{b}	0.98 ± 0.20^{b}
AMN (mg kg^{-1} soil)	69.0 ± 12.2^{a}	116 ± 13.7^{a}	104 ± 15.2^{a}	96.0 ± 8.27^{a}	97.8 ± 30.0^{a}

Electrical conductivity (EC). Phospholipid Fatty Acid Analysis (PLFA). Anaerobically mineralization organic carbon (AMOC). Anaerobically mineralization nitrogen (AMN).

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882 **Table 2.** Spearman's rank correlation coefficients and *P*-values between soil
883 physicochemical properties and abundance of functional genes (gene copies μg^{-1} DNA).
884 Significant correlations are shown in bold.

Functional genes	Bacterial *16SrRNA*	Archaeal *16SrRNA*	Fungal *ITS*	*nifH*	Bacterial *amoA*	Archaeal *amoA*	*nirK*	*nirS*	*nosZ*
pH	**0.478**	0.131	0.071	-0.055	0.066	**0.614**	0.275	**0.515**	**0.495**
p-value	**0.033**	0.583	0.788	0.833	0.801	**0.009**	0.286	**0.034**	**0.043**
EC	**-0.450**	-0.128	-0.018	-0.151	-0.170	-0.471	**-0.522**	**-0.522**	**-0.627**
p-value	**0.047**	0.590	0.944	0.563	0.513	0.057	**0.031**	**0.031**	**0.007**
Bulk density	-0.040	**-0.568**	0.440	**-0.699**	0.419	**0.723**	-0.450	0.368	0.184
p-value	0.867	**0.009**	0.077	**0.002**	0.094	**0.001**	0.070	0.146	0.480
Total porosity	0.043	**0.562**	**-0.427**	**0.704**	-0.414	**-0.726**	0.454	-0.365	-0.168
p-value	0.856	**0.010**	**0.087**	**0.002**	0.098	**0.001**	0.067	0.150	0.519
Soil water content	-0.057	0.378	-0.249	**0.592**	-0.215	**-0.907**	0.215	-0.407	-0.316
p-value	0.810	0.101	0.335	**0.012**	0.408	**0.000**	0.408	0.105	0.216
Organic matter	-0.171	0.338	-0.218	**0.597**	-0.244	**-0.907**	0.261	-0.421	-0.360
p-value	0.471	0.144	0.400	**0.011**	0.345	**0.000**	0.311	0.093	0.155
NH_4^+-N	0.135	0.427	-0.108	**0.582**	-0.195	**-0.669**	0.297	-0.387	-0.333
p-value	0.571	0.060	0.680	**0.014**	0.453	**0.003**	0.247	0.125	0.191
NO_3-N	-0.236	-0.237	0.400	-0.173	-0.147	0.071	-0.387	-0.240	-0.184
p-value	0.317	0.314	0.112	0.507	0.573	0.786	0.124	0.352	0.480
Available P	0.103	**0.485**	-0.081	**0.457**	0.129	**-0.618**	0.116	-0.166	-0.218
p-value	0.665	**0.030**	0.757	**0.065**	0.622	**0.008**	0.656	0.525	0.400
Total C	-0.161	0.407	-0.294	**0.577**	-0.258	**-0.869**	0.253	-0.412	-0.440
p-value	0.497	0.075	0.252	**0.015**	0.318	**0.000**	0.328	0.100	0.077
Total N	-0.023	0.358	-0.007	**0.795**	-0.300	**-0.632**	**0.490**	-0.317	-0.105
p-value	0.925	0.121	0.978	**0.000**	0.241	**0.006**	**0.046**	0.216	0.687
Dissolved organic C	-0.029	0.343	-0.106	**0.580**	-0.201	**-0.674**	0.200	-0.361	-0.439
p-value	0.902	0.139	0.687	**0.015**	0.439	**0.003**	0.442	0.155	0.078
Total dissolved N	-0.062	0.236	0.058	**0.544**	-0.217	**-0.610**	0.201	-0.374	-0.341
p-value	0.796	0.317	0.826	**0.024**	0.403	**0.009**	0.439	0.139	0.181
Microbial biomass PLFA	-0.026	0.276	-0.044	**0.639**	-0.229	**-0.806**	0.256	-0.373	-0.203

p-value	0.912	0.238	0.866	**0.006**	0.376	**0.000**	0.321	0.140	0.434
AMOC	-0.229	-0.263	0.314	0.256	-0.294	-0.181	-0.009	-0.276	0.108
p-value	0.331	0.263	0.220	0.321	0.252	0.486	0.974	0.283	0.680
AMN	-0.033	0.057	0.171	**0.611**	-0.181	**-0.544**	0.316	-0.222	-0.049
p-value	0.890	0.810	0.513	**0.009**	0.486	**0.024**	0.216	0.392	0.852

Electrical conductivity (EC). Phospholipid Fatty Acid Analysis (PLFA). Anaerobically mineralizable organic carbon (AMOC).

888 **Table 3.** Spearman's rank correlation coefficients and *P*-values between soil
889 physicochemical properties and N_2O emission (mg N kg^{-1} h^{-1}) in unamended soil (control)
890 or after the addition of labile C and N.

Soil property	N_2O emissions (Control)	N_2O emissions (C addition)	N_2O emissions (N addition)	N_2O emissions (C and N addition)
Water content	0.24	-0.80	-0.71	-0.81
p-value	0.316	<0.001	<0.001	<0.001
Bulk density	-0.33	0.73	0.70	0.79
p-value	0.152	<0.001	0.001	<0.001
Total nitrogen	0.19	-0.89	-0.65	-0.74
p-value	0.431	<0.001	0.002	<0.001
Total porosity	0.33	-0.74	-0.69	-0.80
p-value	0.152	<0.001	0.001	<0.001
AMOC	0.31	-0.86	-0.70	0.82
p-value	0.179	<0.001	0.001	<0.001

891 Anaerobically mineralizable organic carbon (AMOC).

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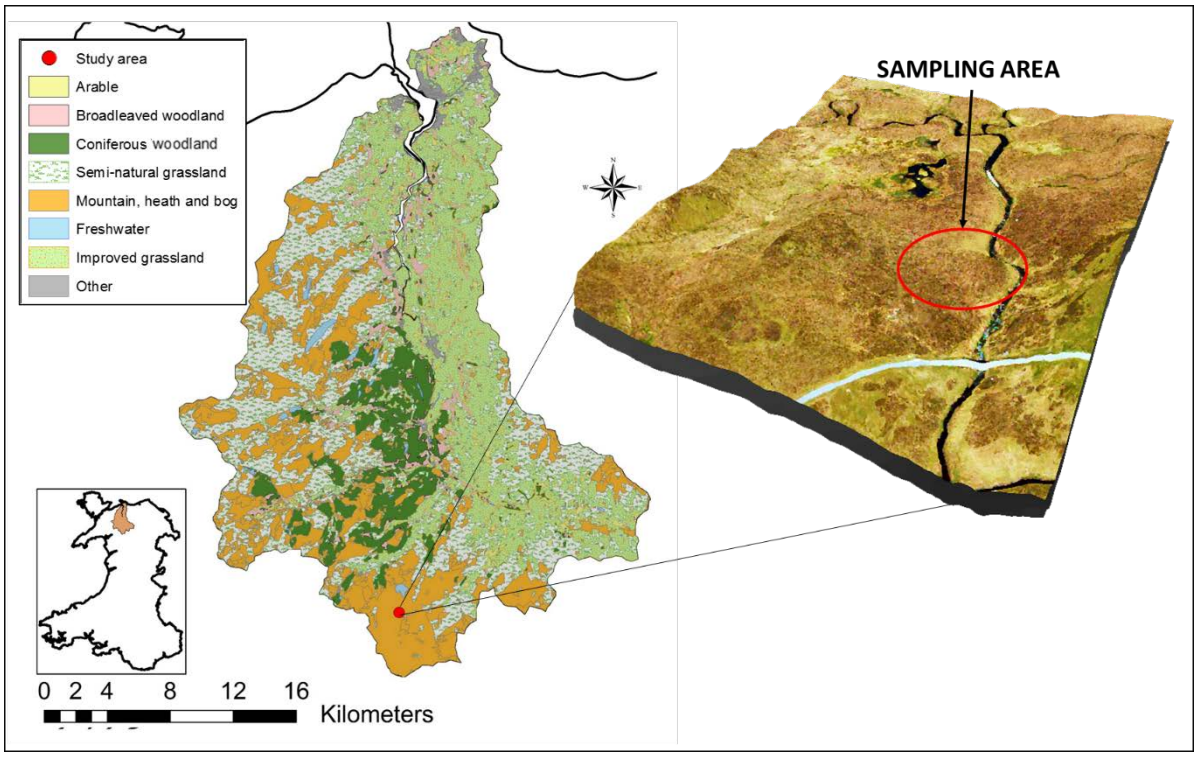

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894 **Fig. 1.** The Conwy catchment, North Wales, UK showing the location of the riparian
895 sampling area and the major land cover classes.

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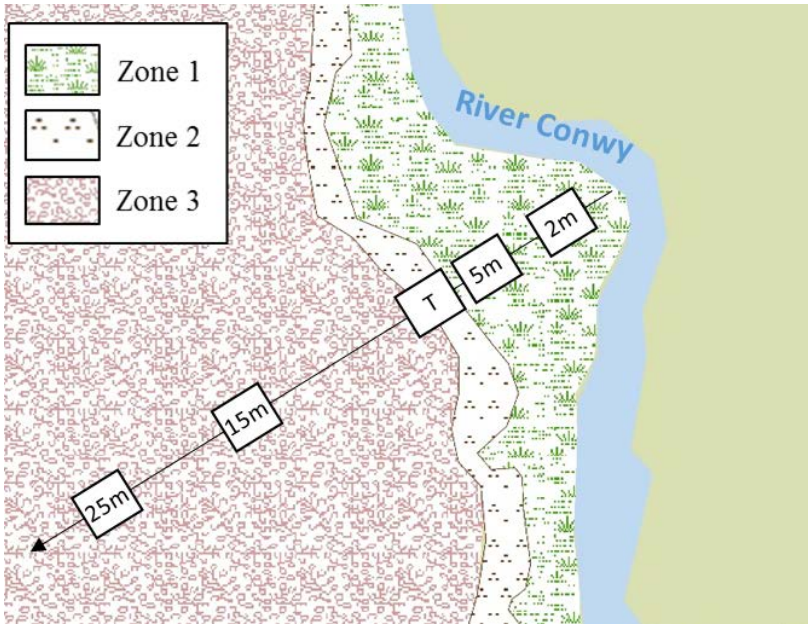


Fig. 2. Location of sample points across the riparian area. Different colours indicate changes in vegetation. Zone 1 represents the area dominated by *Juncus effusus*, Zone 2 corresponds to the transitional area between the grasses and the heath, and Zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species.

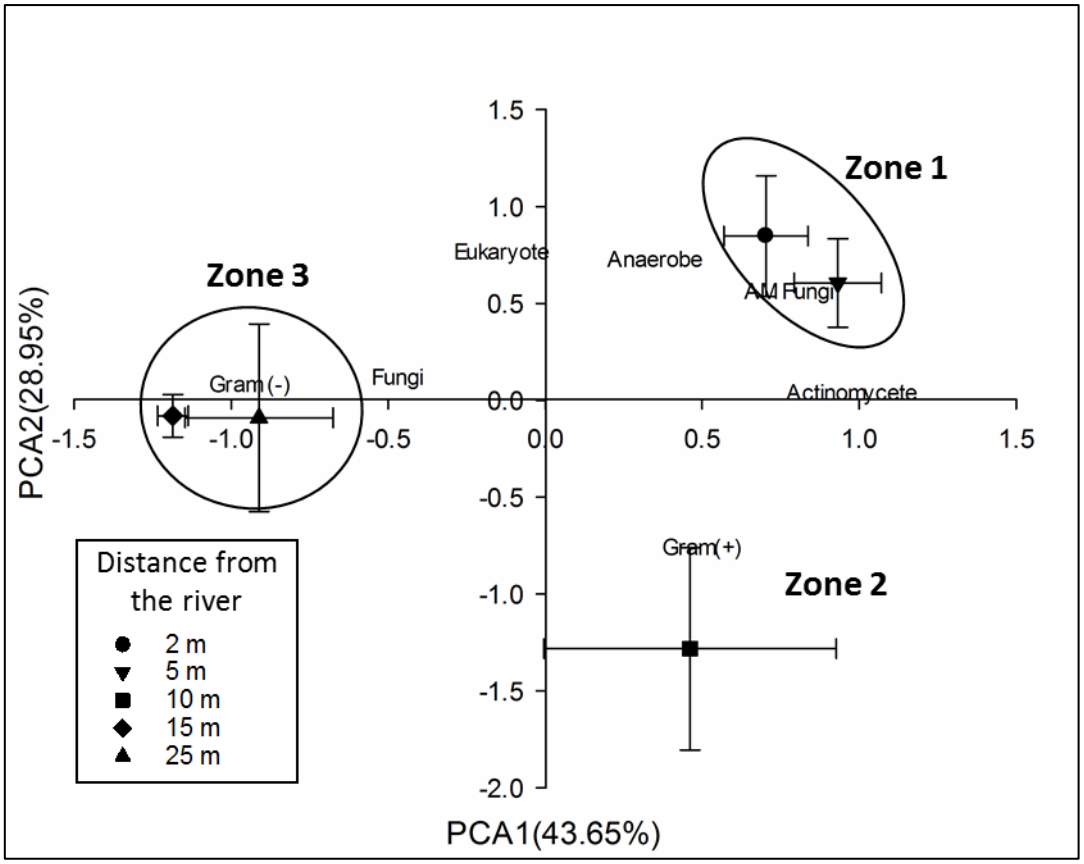


Fig. 3. Correlation bi-plot from the principal component analysis (PCA) on PLFA microbial groups (%) with respect to distance from the river (n = 4). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (2 and 5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m), and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (15 and 25 m). Correlation of PLFA microbial groups with the main axes are given by their specific names and distance from the river by cluster centroids (average score on each horizontal principal component (PC1) and vertical principal component (PC2) with standards errors). Circles represents sample points within the same zone.

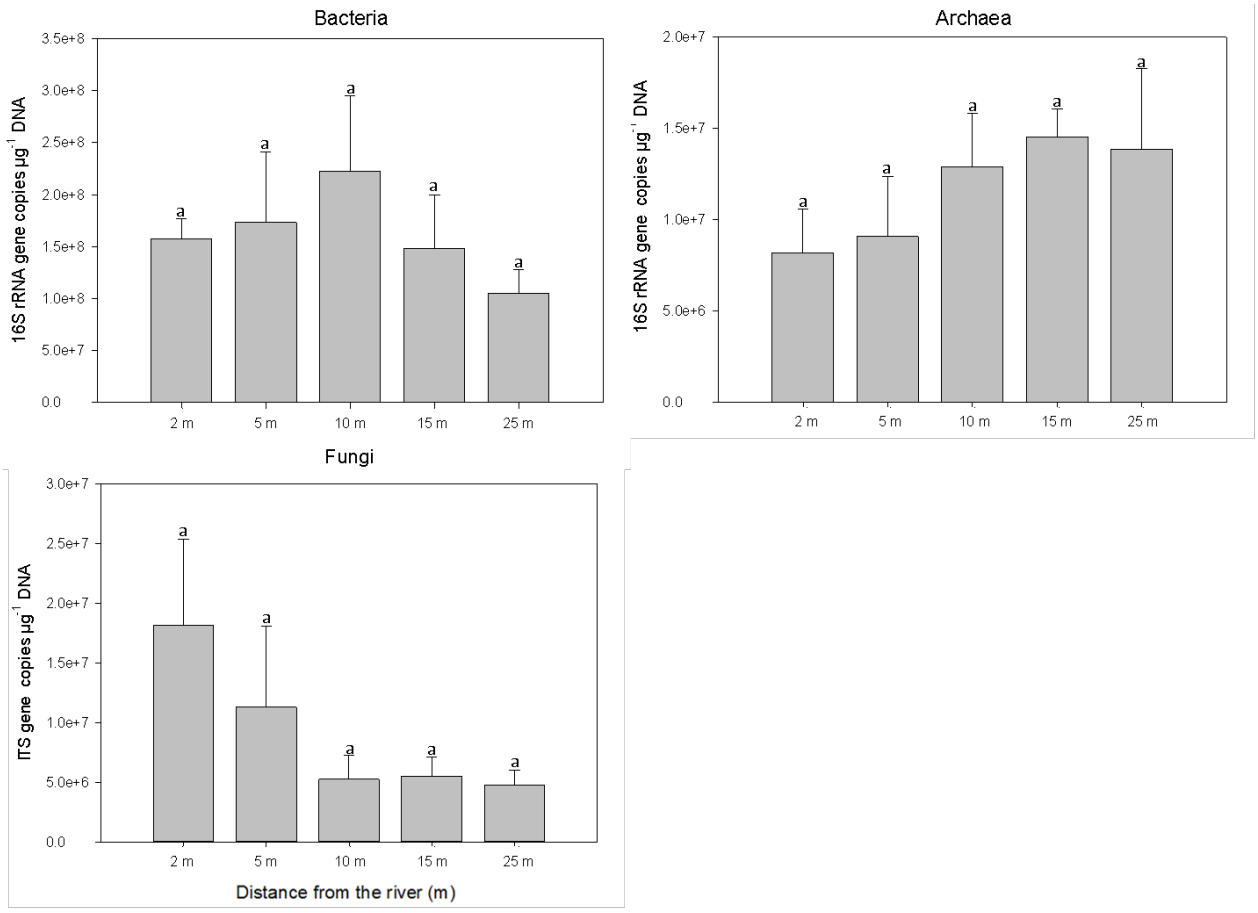


Fig. 4. Total bacterial, archaeal and fungal gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences ($P > 0.05$) with respect to distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values ($n = 4$ for 10, 15 and 25 m, $n = 3$ for 2 m and $n = 2$ for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 2.

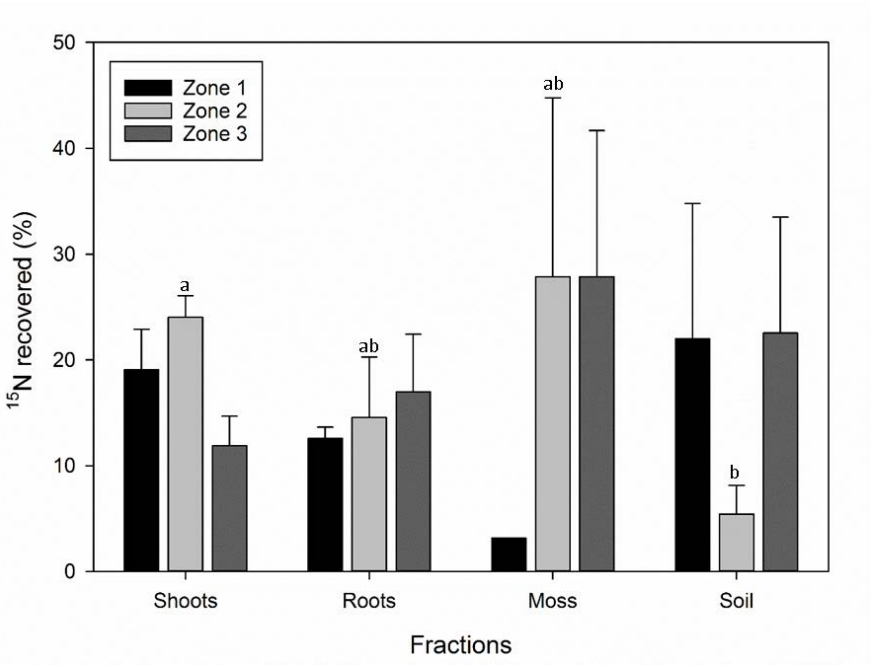
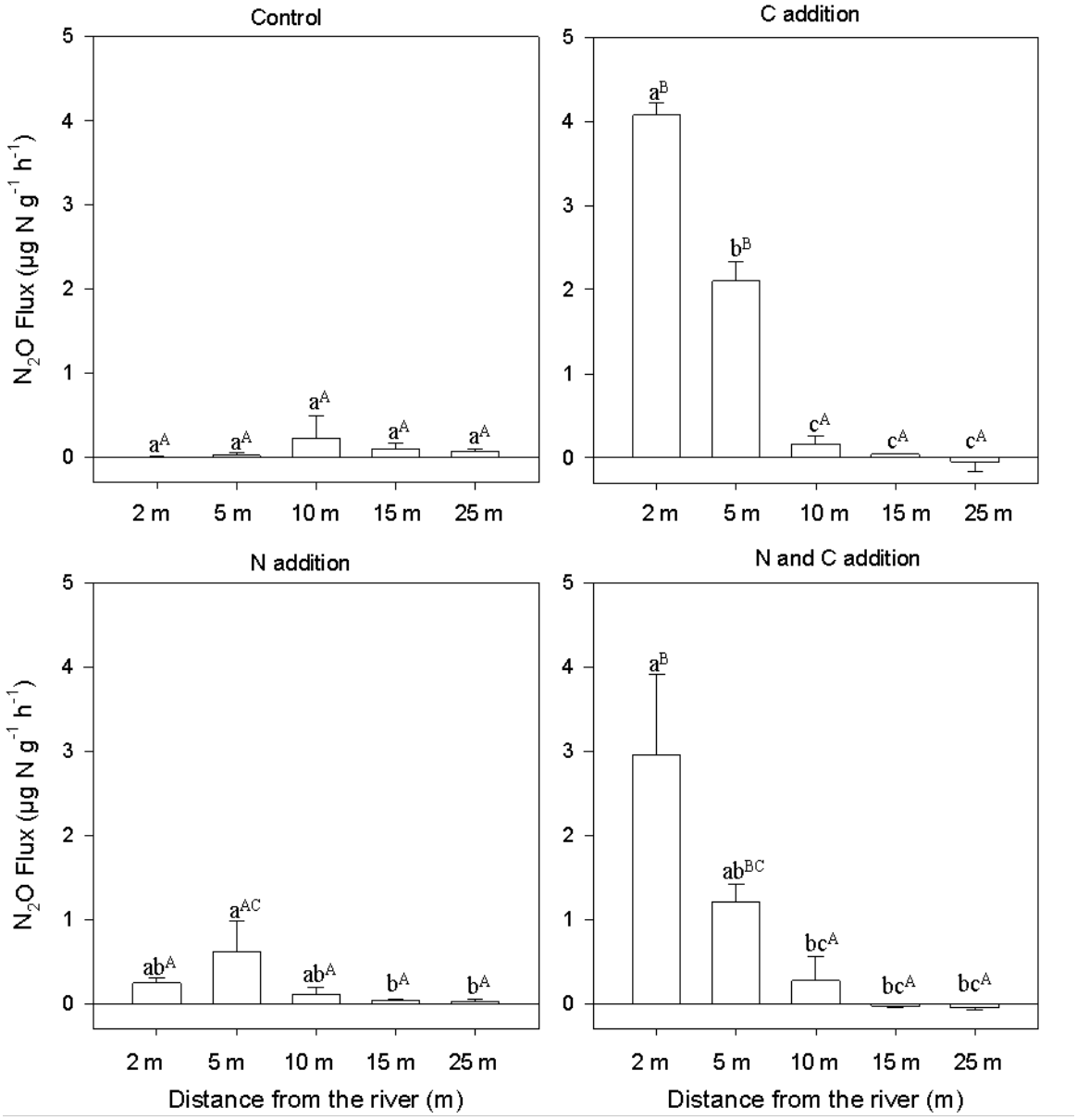


Fig. 5. Recovery of ^{15}N (% of total applied) from within the different fractions (shoots, roots, mosses and soil) represented by bars (n = 3 except moss in zone 1 where n = 1). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m) and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (25 m). Same lower case letters or the lack of it indicate no significant differences (P > 0.05) with respect to the different fractions within each zone according to one-way ANOVA and the Tukey post-hoc test.



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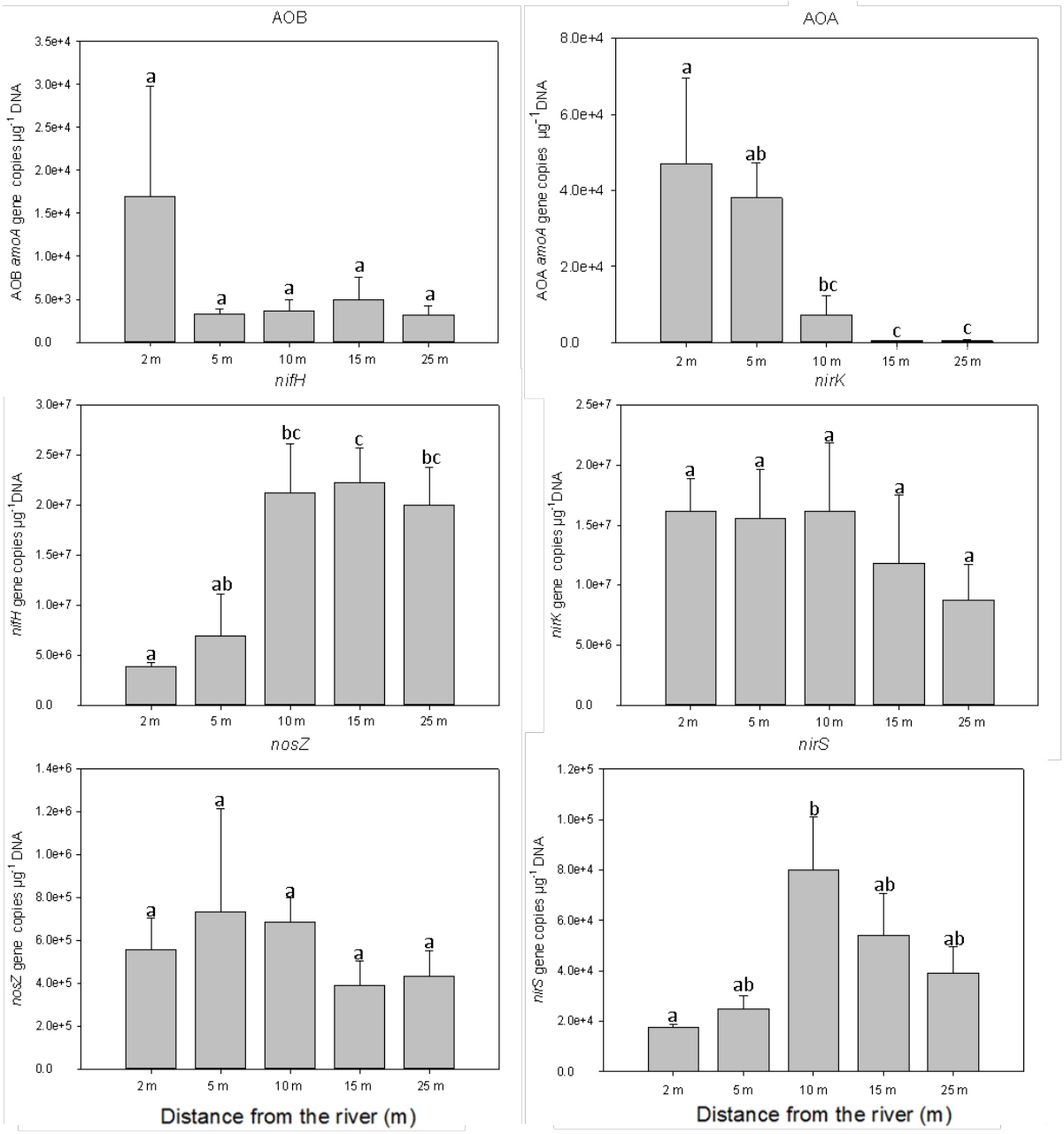

Fig. 7. Bacterial *amoA* (AOB), archaeal *amoA* (AOA), *nifH*, *nirS*, *nosK*, *nosZ* gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences ($P > 0.05$) relative to distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values ($n = 4$ for 10, 15 and 25 m, $n = 3$ for 2 m and $n = 2$ for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 2.

Spatial zoning of microbial function and plant-soil nitrogen dynamics across a riparian area

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Supplementary on-line information

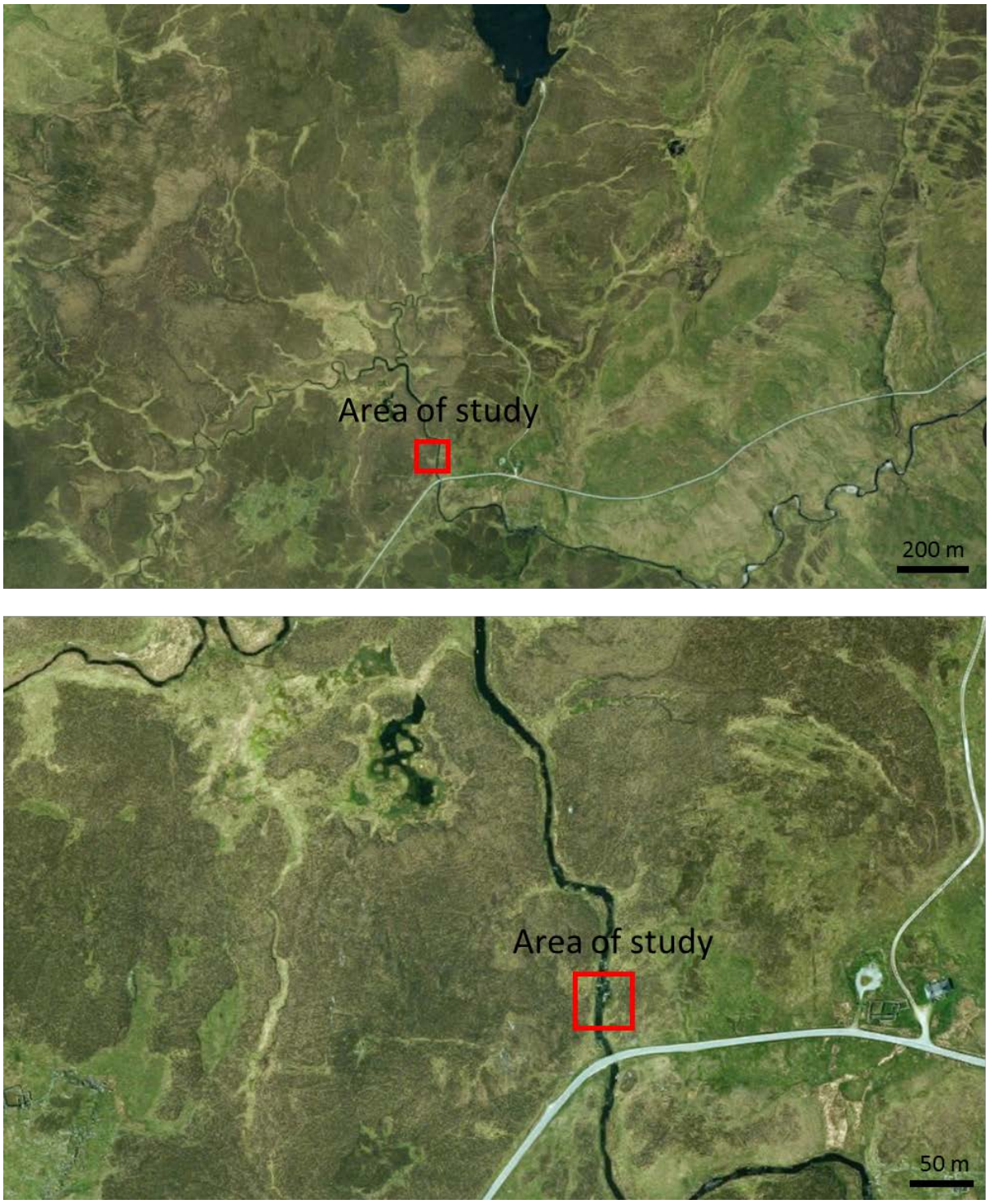

Fig. S1. Aerial photography of the area of study.

Fig. S2. Detailed photographs of vegetation in the area of study.

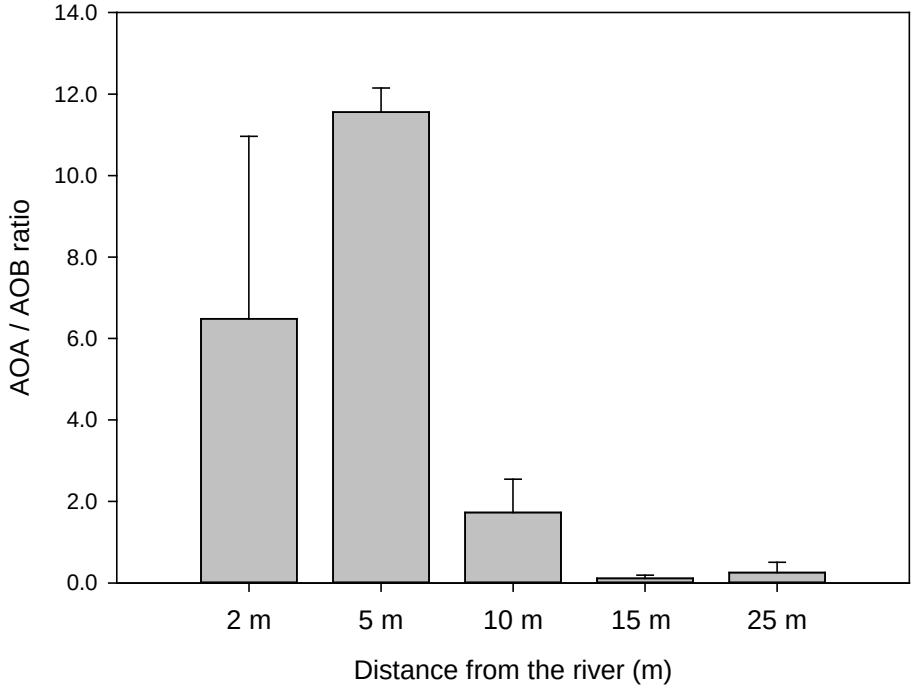

Fig. S3. Ratios of AOA to AOB *amoA* copy numbers relative to distance from the river. Bars represent mean values (n = 4 for 2, 10, 15 and 25 m and n = 2 for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 3.

Table S1. PLFA biomarkers used for taxonomic microbial groups

Microbial group category	PLFA specific fatty acids			
AM Fungi	16:1 w5c			
Saprophytic Fungi	18:2 w6c			
Gram Negative	10:0 2OH	14:0 2OH	18:1 w6c	21:1 w8c
	10:0 3OH	16:1 w9c	18:0 cyclo w6c	21:1 w6c
	12:1 w8c	16:1 w7c	18:1 w3c	21:1 w5c
	12:1 w5c	16:1 w6c	19:1 w9c	21:1 w4c
	13:1 w5c	16:1 w4c	19:1 w8c	21:1 w3c
	13:1 w4c	16:1 w3c	18:1 w5c	22:1 w9c
	13:1 w3c	17:1 w9c	19:1 w6c	22:1 w8c
	12:0 2OH	17:1 w8c	19:0 cyclo w9c	22:1 w6c
	14:1 w9c	17:1 w7c	19:0 cyclo w7c	22:1 w5c
	14:1 w8c	17:1 w6c	9:1 w17c	22:1 w3c
	14:1 w7c	17:1 w5c	20:1 w9c	22:0 cyclo w6c
	14:1 w5c	17:1 w4c	20:1 w8c	24:1 w9c
	15:1 w9c	17:1 w3c	20:1 w6c	24:1 w7c
	15:1 w8c	16:0 2OH	19:0 cyclo w6c	11:0 iso 3OH
	15:1 w7c	17:0 cyclo w7c	20:1 w4c	14:0 iso 3OH
	15:1 w6c	18:1 w8c	20:0 cyclo w6c	
	15:1 w5c	18:1 w7c	21:1 w9c	
Methanotroph	16:1 w8c			
Eukaryote	15:4 w3c	19:3 w3c	22:5 w6c	23:3 w3c
	15:3 w3c	20:4 w6c	22:6 w3c	23:1 w5c
	16:4 w3c	20:5 w3c	22:4 w6c	23:1 w4c
	16:3 w6c	20:3 w6c	22:5 w3c	24:4 w6c
	18:3 w6c	20:2 w6c	22:2 w6c	24:3 w6c
	19:4 w6c	21:3 w6c	23:4 w6c	24:3 w3c
	19:3 w6c	21:3 w3c	23:3 w6c	24:1 w3c
Gram Positive	11:0 iso	14:0 iso	16:0 iso	17:1 anteiso w7c
	11:0 anteiso	14:0 anteiso	16:0 anteiso	19:0 iso
	12:0 iso	15:1 iso w9c	17:1 iso w9c	19:0 anteiso
	12:0 anteiso	15:1 iso w6c	17:0 iso	20:0 iso
	13:0 iso	15:1 anteiso w9c	17:0 anteiso	22:0 iso
	13:0 anteiso	15:0 iso	18:0 iso	
	14:1 iso w7c	15:0 anteiso	17:1 anteiso w9c	
Anaerobe	12:0 DMA	15:0 DMA	16:1 w5c DMA	18:1 w7c DMA
	13:0 DMA	16:2 DMA	16:0 DMA	18:1 w5c DMA
	14:1 w7c DMA	17:0 DMA	18:2 DMA	18:0 DMA
	14:0 DMA	16:1 w9c DMA	18:1 w9c DMA	
	15:0 iso DMA	16:1 w7c DMA		
Actinomycetes	16:0 10-methyl	18:1 w7c 10-methyl	22:0 10- methyl	
	17:1 w7c 10-methyl	18:0 10-methyl	20:0 10- methyl	
	17:0 10-methyl	19:1 w7c 10-methyl		

Table S2. List of the primers used to target each community.

Target gene	Primer	Sequence 5'-3'	References
Bacterial *16SrRNA*	341F	CCT AYG GGR BGC ASC AG	Glarling et al. (2015)
	806R	GGA CTA CNN GGG TAT CTA AT	
Archaeal *16SrRNA*	Parch519F	CAG CMG CCG CGG TAA	Øvreaset al. (1997)
	Arch1060R	GGC CAT GCA CCW CCT CTC	Reysenbach and Pace, (1995)
Fungal *ITS*	ITS1f	TCC GTA GGT GAA CCT GCG G	Gardes and Bruns (1993); Vilgalys and Hester (1990)
	5.8s	CGC TGC GTT CTT CAT CG	
nifH	PolF	TGC GAY CCS AAR GCB GAC TC	Poly et al. (2001)
	PolR	ATS GCC ATC ATY TCR CCG GA	
amoA Bacteria	amoA-1F	GGG GTT TCT ACT GGT GGT	Rotthauwe et al. (1997)
	amoA-2R	CCC CTC KGS AAA GCC TTC TTC	
amoA Archaea	Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Francis et al. (2005)
	Arch-amoAR	GCG GCC ATC CAT CTG TAT GT	
nirK	nirK876F	ATY GGC GGV CAY GGC GA	Henry et al. (2004)
	nirK1040R	GCC TCG ATC AGR TTR TGG TT	
nirS	cd3aF	GTS AAC GTS AAG GAR ACS GG	Throbäck et al. (2004)
	R3cdR	GAS TTC GGR TGS GTC TTG A	
nosZ	nosZ1F	CGC RAC GGC AAS AAG GTS MSS GT	Henry et al. (2006)
	nosZ1R	CAK RTG CAK SGC RTG GCA GAA	
nosZII	nosZ-II-F	CTI GGI CCI YTK CAY AC	Jones et. al (2013)
	nosZ-II-R	GCI GAR CAR AAI TCB GTR C	

Table S3. Phospholipid fatty acid (PLFA) ratios of main microbial groups. Values represent means ± SEM (n = 4). Same lower case letters indicate no significant differences (P > 0.05) with respect to distance from the river according to one-way ANOVA and the Tukey post-hoc test.

PLFA ratio	Zone 1		Zone 2	Zone 3	
	2 m	5 m	10 m	15 m	25 m
Fungi/Bacteria	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.04 ± 0.004^{b}	0.03 ± 0.005^{b}	0.04 ± 0.006^{b}
Predator/Prey	0.03 ± 0.003^{a}	0.03 ± 0.001^{a}	0.02 ± 0.004^{a}	0.03 ± 0.004^{a}	0.03 ± 0.005^{a}
Gram +/Gram -	0.76 ± 0.03^{ab}	0.83 ± 0.04^{b}	0.88 ± 0.10^{b}	0.56 ± 0.01^{c}	0.61 ± 0.03^{ac}
Saturated/Unsaturated	1.01 ± 0.09^{a}	1.05 ± 0.10^{a}	1.38 ± 0.26^{a}	0.74 ± 0.04^{a}	0.88 ± 0.18^{a}
Mono/Poly	13.5 ± 1.18^{a}	14.4 ± 2.68^{a}	15.9 ± 1.76^{a}	17.6 ± 1.50^{a}	16.6 ± 2.44^{a}
16w/16 cyclo	3.31 ± 0.19^{a}	3.89 ± 0.23^{a}	4.11 ± 0.53^{a}	9.65 ± 0.54^{b}	9.20 ± 0.69^{b}
18w/19 cyclo	$0.70 \quad 0.07^{a}$	$0.64 \quad 0.04^{a}$	$0.85 \quad 0.11^{a}$	$1.93 \quad 0.08^{b}$	$2.06 \quad 0.25^{b}$

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